INTERNATIONAL JOURNAL OF
PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

# SPECTROPHOTOMETRIC METHODS FOR THE SIMULTANEOUS DETERMINATION OF SULFADOXINE AND PYRIMETHAMINE IN BULK AND TABLETS FORMS

**MYADA H. MOHAMED, SHAZA W. SHANTIER, ELRASHEED A. GADKARIEM**

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Khartoum, Sudan, P. O. Box 1996.

**Accepted Date: 26/11/2017; Published Date: 27/12/2017**

**Abstract:** Three sensitive and accurate spectrophotometric methods were developed for the quantitative determination of sulfadoxine and pyrimethamine in bulk and tablets forms. Method I based on solvent extraction process, using separatory funnel, which depends on their partition in different solvents and measuring the extracts spectrophotometrically. Sufadoxine was extracted using 1M NaOH (upper layer) and pyrimethamine using $CHCl_3$ (lower layer). The swamping method (method II) was dependent on the presence of high amount of SUL compared to the PYR (20: 1). Method III based on the simultaneous determination of both drugs at 274nm and 286nm. The developed methods were validated according to ICH guidelines in terms of linearity, accuracy and precision.

**Keywords:** Sulfadoxine; Pyrimethamine; Spectrophotometry; Extraction; Validation.

**Corresponding Author: SHAZA W. SHANTIER**

**Access Online On:**

www.ijprbs.com

**How to Cite This Article:**

Shaza W. Shantier, IJPRBS, 2017; Volume 6(6): 16-28

*PAPER-QR CODE*

## INTRODUCTION

Sulfadoxine (SUL; $N^1$-(5, 6-dimethoxy-4-pyrimidinyl) benzene sulfanilamide (figure 1), is a long acting sulfonamide (plasma $t_{1/2}$ 7-9 days) that is absorbed rapidly [1]. It is a bacteriostatic drug that blocks the incorporation of P-aminobenzoic acid to form dihydropteroic acid [2]. Its acidity Belongs to its sulphonamide group which linked to a benzene ring. It dissolves in solutions of alkali hydroxides as NaOH and in dilute mineral acids as HCl [3].

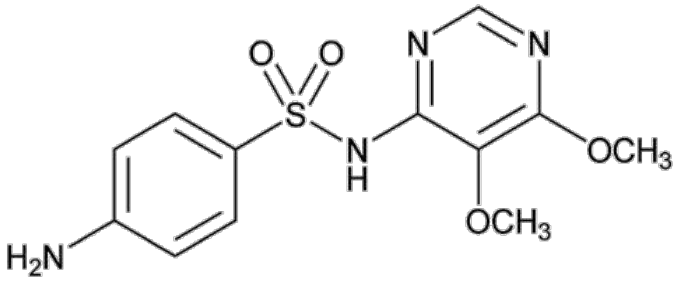


**Figure 1: Chemical structure of SUL.**

Pyrimethamine (PYR), 5-(4-Chlorphenyl)-6-ethylpyrimidine-2, 4-diamine (Figure 2) [3], is a diaminopyrimi-dine derivative related to trimethoprime [4]. It is a slow acting blood schizontocide which is used in combination with sulfadiazine and folinic acid as a first line in treatment of toxoplasmosis, one of the opportunistic infections in HIV AIDS patients [1,4]. It is used with SUL in treatment of malaria to enhance its antifolate activity [1]. It is almost white crystalline powder, practically insoluble in water and in NaOH, soluble in chloroform [3].

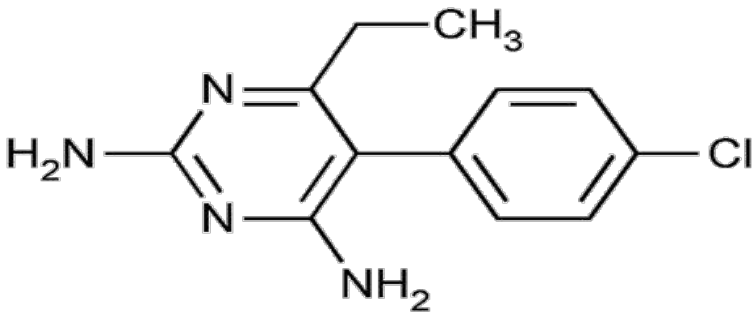


**Figure 2: Chemical structure of PYR.**

SUL and PYR are combined together in the ratio of 20: 1, respectively. It is a folate antagonist combination, which is used in treatment of uncomplicated plasmodium falciparum malaria in many endemic areas especially against chloroquine- resistant strains where combined with artesunate [5].The combination of PYR and SUL inhibits folate biosynthesis in parasite via

inhibition of dihydrofolatereductase (DHFR) by the PYR and inhibition of dihydropteroate synthetase (DHPS) by SUL [1]. This combination has the great advantages of the ease of administration as a single oral dose and low cost [4].

SUL and PYR are officially assayed by HPLC methods [6]. Literature survey revealed different methods for their analysis either alone or in combination or in biological fluids. These methods include mainly HPLC method [2, 7- 12] and spectrophotometric methods [5, 13- 17].

The main aim of this work was to develop and validate fast, simple, precise, sensitive and accurate spectrophotometric method as substitute for chromatographic and other methods reported for the simultaneous determination of PYR and SUL in bulk and dosage form with satisfactory precision

## Materials and Methods

### Instruments and equipments

All spectrophotometric measurements were conducted on Shimadzu UV 1800 (ENG 240 V, Japan). Standards and samples' weighing was made using electronic balance (Kern ALS120-4, Germany).

### Standards and Samples

Both SUL (99.89%) and PYR (99.69%) were kindly provided by Amipharma Laboratories, Sudan.

Two different brands (B1, 2) of combined SUL and PYR tablets (SUL 500mg+ PYR 25mg/tablet) were purchased from local pharmacies;

### Chemicals

Sodium hydroxide 1M (NaOH), Oxford Lab Chem., India. Methanol ($CH_3OH$), Chem-Lab NV, Belgium. Chloroform ($CHCl_3$), Carlo Ebra Reagents, Spain. Sodium sulphate anhydrous Purified LR (for synthesis) ($Na_2SO_4$), SdfiNE- CHEM LimiTED, Mumbai.

### Preparation of stock solutions

### SUL standard solution

SUL standard (0.025g) was accurately weighed and dissolved in 50ml of 1M NaOH. 5ml of this solution was transferred into 50ml volumetric flask and diluted up to mark by NaOH (Solution A: 50µg/ml).

**PYR standard solution**

PYR standard (0.05g) was accurately weighed and dissolved in 50ml $CHCl_3$. 5 ml of this solution was transferred into 50 ml volumetric flask and diluted up to mark by $CHCl_3$ (Solution C; 100µg/ml).

**Synthetic standard mixture solution**

SUL and PYR standards (0.1g and 0.005g, respectively) were accurately weighed and transferred into a separatory funnel. 50ml of 1M NaOH was added and shaken for 30 minutes. Then 40 ml of $CHCl_3$ was added and the mixture was shaken slightly. Another 10ml of $CHCl_3$ was added to complete its volume to 50ml. The mixture was allowed to stand for few minutes. The organic layer ($CHCl_3$) was then separated and dried using sodium sulphate anhydrous as drying agent and then filtered; (PYR1solution; 100µg/ml). Another 50ml of 1M NaOH was added to the upper layer to complete the volume to 100ml. 5ml of this solution was transferred into 100ml volumetric flask and the volume was completed with 1M NaOH; (SUL1solution; 50µg/ml).

**Sample stock solution**

For each brand, an amount of finely powdered tablet equivalent to 0.1g SUL and 0.005g PYR was accurately weighed and transferred into a separatory funnel. The powder mixture was treated as under standard solution and the resultant solutions were then filtered to obtain PYR2 solution: 100µg/ml and SUL2 solution; 50µg/ml.

**Procedure**

**Determination of λmax**

Standard solutions of SUL and PYR were scanned in the range 200-400nm to establish the wavelength maxima for each.

**Construction of calibration curves**

Aliquots of solution A (1-5ml) were transferred into a set of 25ml volumetric flasks and the volumes were completed to mark with 1M NaOH to obtain the concentrations 2-10 µg/ml. Serial volumes of solution B (1-5ml) were transferred into a set of 50ml volumetric flasks and treated as solution A using $CHCl_3$ as diluents. The absorbance values of the resultant solutions were measured at established $\lambda_{max}$271nm and 288nm against 1MNaOH and $CHCl_3$ as blanks, respectively. Calibration curves were constructed by plotting the corresponding absorbance values against the concentrations for each drug. This procedure was repeated three times and the regression analysis data was calculated.

**Sample assay**

Three ml of either SUL2orPYR2 solution for each brand was treated under calibration curve and the content% (w/v) for each drug was then calculated.

**Method validation:**

The validity of the method was determined according to ICH guidelines [18], where linearity, LOD, LOQ, accuracy and precision were determined.

**Linearity**

Slope (b), intercept (a), correlation coefficient ($r^2$) and residual sum of squares were calculated from the constructed calibration curves.

**Limit of Detection and Limit of Quantitication**

LOD and LOQ were calculated from the calibration curve according to the formulae [19]:

$$L.O.D = \frac{3.3Sy/x}{b} \quad \text{and} \quad L.O.Q = \frac{10\,Sy/x}{b}$$

Where *L.O.D* is the limit of detection, LOQ is the limit of quantification, $Sy/x$ is the standard deviation of the response and *b* is the slope of the calibration curve.

**Precision**

Aliquots of either SUL1 or PYR1 solution (2, 3 and 4ml) were treated as under calibration curve. The procedure was repeated three times within the same day and between days to evaluate the repeatability and reproducibility of the developed method. RSD% was then calculated from the mean and SD values.

**RESULTS AND DISCUSSION**

In chemical analysis the use of instrumental methods normally suffer from interferences. Drug combinations or their impurities need special manipulation to solve the problem of interference with the assay of drugs.

Nowadays, HPLC has become the method of choice in such cases; however, HPLC is considered an expensive and time consuming method. Resorting to the simple available UV spectrophotometric method is an alternative if the problem of interference can be overcome. In the present work, we planned to determine both drug simultaneously taking into account their different solubility properties.

**Solvent extraction method**

Suitable dilutions were made in 1M NaOH and $CHCl_3$ for SUL and PYR, respectively. SUL solution in 1MNaOH showed absorption maxima at 271nm while PYR in $CHCl_3$ was recorded at 288nm (Figures 3, 4). The synthetic standard mixture was prepared to resemble the sample concentration. SUL1 and PYR1 spectra showed identical wavelength maxima and absorption values to the pure standard. This result reflect the feasibility of the proposed method and hence its application for sample determination.

Constructed calibration curves for both drugs (Figure 5) obeyed the Beer's law in the concentration range 2-10µg/ml with good correlation coefficient (not less than 0.999). Sensitivity of the developed method was reflected by the low values of slope, LOD and LOQ. Regression analysis data are presented in table 1

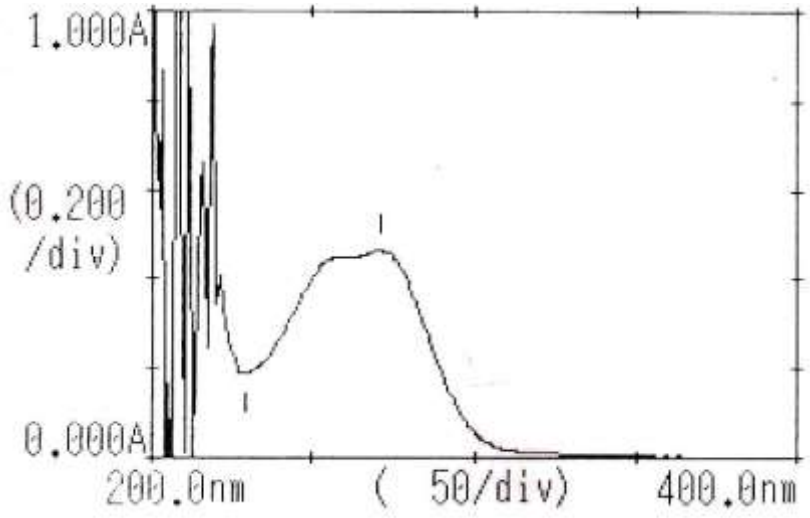


**Figure 3: Spectrum of SUL standard solution in 1M NaOH**.

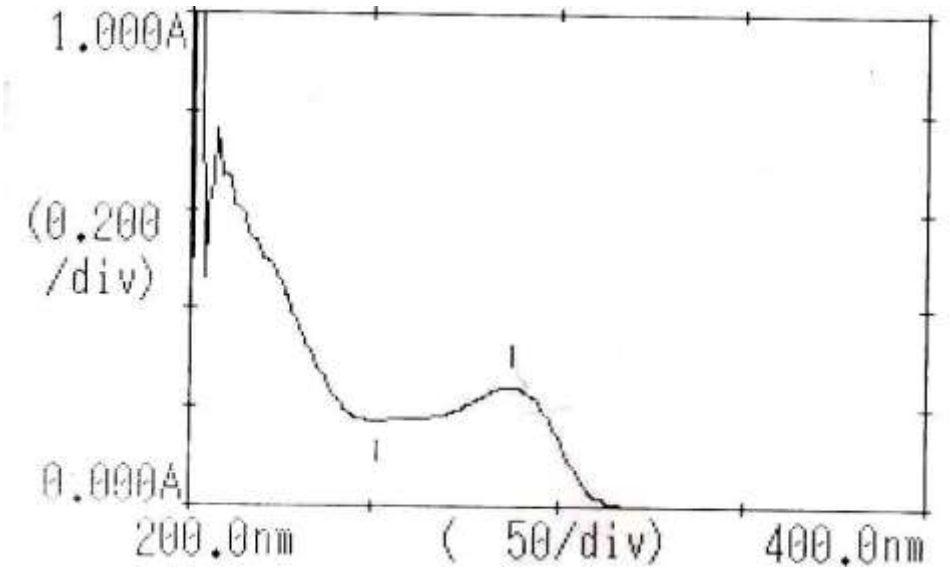


**Figure 4**: **Spectrum of PYR standard solution in $CHCl_3$.**

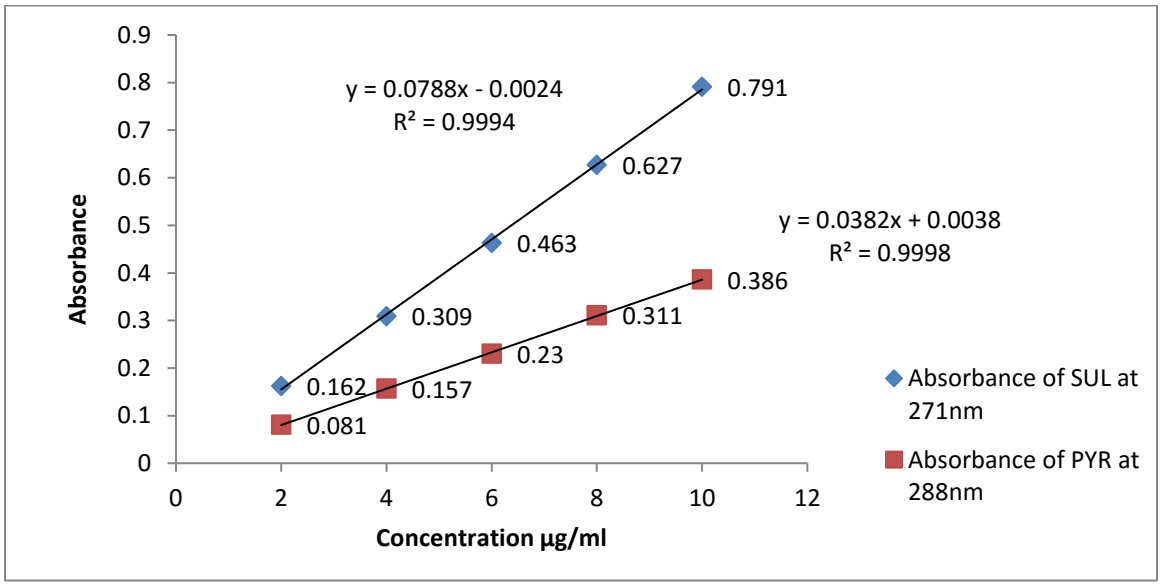


**Figure 5: SUL and PYR standard calibration curves (SUL/ 1M NaOH, PYR/ $CHCl_3$).**

**Precision**

The precision of the developed methods was evaluated by the results obtained from between-days (reproducibility) and within-day data (repeatability) for three concentrations within the linearity range. The calculated RSD values for both drugs were found to be within the accepted limit (less than 2%, Table 2).

**Tablets content**

Finally, the proposed method was applied for the determination of SUL and PYR in the tablet dosage form. The mean content percent of three independent analyses was determined and summarized in table 3.

The validity of the method was then assessed by calculating t-value according to the following equation[19]

$$t = (\bar{x} - \mu)\sqrt{n}/SD$$

Where $\bar{x}$ is sample mean, *SD* is sample standard deviation, $\mu$ is true mean and *n* is sample size.

**Simultaneous determination**

SUL and PYR were found soluble to the same extent in methanol. Spectrum of standard mixture prepared in methanol is shown in Figure 6.

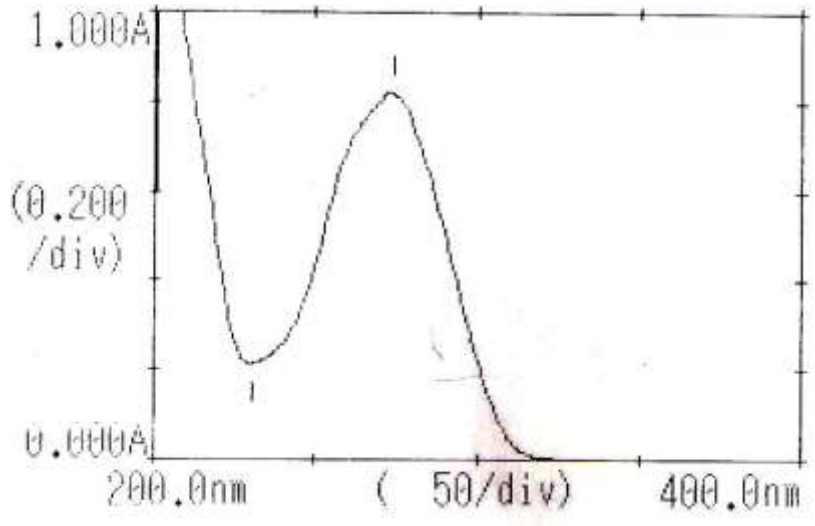


**Figure 6: Spectrum of SUL and PYR standard mixture in $CH_3OH$ (10: 0.5µg/ml)**

This finding lead to the idea of determining SUL and PYR simulataneously as they overlap at the wavelength 274nm and 286nm. Specific absorptivities were calculated for pure SUL and PYR and then their concentrations in the tablets were determined using the following equations:

$A_1 = A_{x1} + A_{y1} = A^{1\%}_{1cm\,x1} C_x + A^{1\%}_{1cm\,y1} C_y$ → at 274nm

$A_2 = A_{x2} + A_{y2} = A^{1\%}_{1cm\,x2} C_x + A^{1\%}_{1cm\,y2} C_y$ → at 286nm

Where;

$A_1$ and $A_2$ are absorbance values at λ1 and λ2 for the mixture. $A_{x1}$ and $A_{y1}$, $A_{x2}$ and $A_{y2}$ are absorbance values for SUL and PYR at 274nm and 286nm.$A^{1\%}_{1cmx1}$, $A^{1\%}_{1cmy1}$, $A^{1\%}_{1cmx2}$ and $A^{1\%}_{1cmy2}$ are the specific absorptivities for SUL and PYR at λ1 and 2. *C*X and *C*Y are the concentrations of SUL and PYR, respectively. The obtained results are summarized in Table 4.

**Swamping method**

In application, swamping is dependent on the presence of two compounds, one at high concentration and the other is at low concentration; both could be of high absorbance value. Dilution of such compounds mixture will lead to cancelling the absorbance of the low concentration compound.

SUL and PYR are found in the ratio 20:1, therefore by carrying dilution the absorption by PYR will be removed leaving only the absorbance of SUL. This was achieved by scanning the diluted standard mixture of SUL and PYR (5: 0.25 µg/ml) in the range 200nm to 400nm.The only obtained maximum absorbance was at 274nm which is for SUL without interference with PYR (Figure 7).

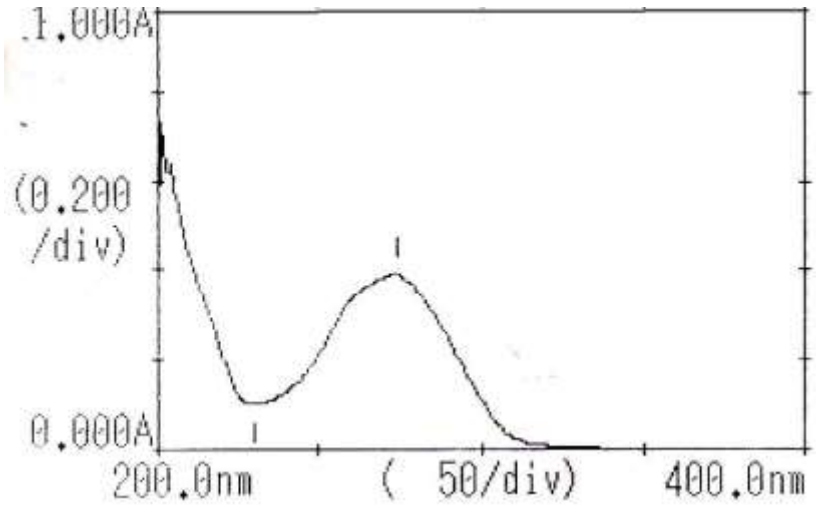


**Figure 7: Spectrum of SUL and PYR standards mixture in $CH_3OH$ (5µg/ml; 274nm)**

This method was validated and then applied for the assay of SUL in the two commercial brands. The results obtained are summarized in Table 5. Results reflect good accuracy and precision.

## CONCLUSION

The developed methods were able to quantify SUL and PYR content simultaneously in bulk and dosage forms with sufficient accuracy and precision. They are considered suitable for the routine quality control analysis of SUL/ PYR tablets dosage form.

**Table 1: Regression analysis data of the developed method**

| Parameter | SUL | PYR |
|---|---|---|
| Wavelength nm | 271 | 288 |
| Range (µg/ml) | 2 -10 | 2 -10 |
| Slope b ± $ts_b$ | 0.0788 ± 0.0035 | 0.038 ± 0.00102 |
| Intercept a ± $ts_a$ | -0.0024 ± 0.023 | 0.0038 ± 0.0068 |
| L.O.D (µg/ml) | 0.292 | 0.176 |
| L.O.Q (µg/ml) | 0.885 | 0.534 |
| $R^2$ | 0.9994 | 0.9998 |

**Table 2: Precision of the developed methods (n=3)**

| | Repeatability | | | Reproducibility | | |
|---|---|---|---|---|---|---|
| **SUL** | 0.2 | 0.2 | 0.2 | 0.6 | 0.2 | 0.5 |
| **PYR** | 0.3 | 0.4 | 0.3 | 1.4 | 0.7 | 1.0 |

**Table 3: SUL and PYR mean content percents for B1-2 (n=3) and validation results**

| Brand | | Conent% ± SD | t tab (t cal)* |
|---|---|---|---|
| **1** | SUL | 99.50 ± 0.82 | 3.01 (4.3) |
| | PYR | 98.92 ± 0.43 | 0.38 (4.3) |
| **2** | SUL | 98.11 ± 0.25 | 0.40 (4.3) |

| | | | |
|---|---|---|---|
| | PYR | 97.25 ± 1.74 | 0.34 (4.3) |

*Calculated at 95% confidence limit for 2 degrees of freedom

**Table 4. Simultaneous determination Results**

| λ | $A_{1cm}^{1\%}$ | | Conc. in brand 1 | | Conc. in brand 2 | |
|---|---|---|---|---|---|---|
| | SUL | PYR | SUL | PYR | SUL | PYR |
| **274nm** | 722 | 318 | 10.4 | 0.49 | 10.26 | 0.55 |
| **286nm** | 540 | 377 | | | | |

**Table 5. Swamping method results**

| | Precision, RSD%, n= 3 | Accuracy, n=4 (t cal; t tab) | Assay (%±SD, n=3) |
|---|---|---|---|
| **SUL, 5µg/ml** | 0.15 – 0.39 | 1.233; 3.18 | 99.32 ± 1.47 |

**REFERENCES**

1. Laurance L. Brunton, Bruce A. Chabner, Bjorn C. Knollman, The Pharmacological basis of therapeutics, Goodman and Gilman's, 12th edition, p: 1377- 1467.

2. Saeed Arayne M, Najma S, Farhan AS, Sajida N, Faiza Q. Simultaneous determination of pyrimethamine, sulfadoxine, mefloquine, and ibuprofen in pharmaceutical formulations by RP-HPLC. Med. Chem. Res. 2010; 19 (9): 1043-1054.

3. European Pharmacopoeia 5.0. 2005. p. 2343-2507

4. Betram G. Katzung, Susan B. Masters, Anthony J. Trevor, Basic and Clinical Pharmacology, 11th edition, 2009; p: 899- 909.

5. Patel K.N., Patel J.K., Patel J.D. Simultaneous Spectrophtometric determination of pyrimethamine and mefloquine hydrochloride in pharmaceutical formulations, International Journal for Research in Management and Pharmacy, 2012; 1 (1).

6. United States Pharmacopoeia/The National Formulary (USP30/NF25), p 3243.

7. Berzas J J, Castaneda G, Guzman F J. Simultaneous determination of sulfaquinoxaline, sulfamethazine and pyrimethamine by liquid chromatography. J Chromatogr A, 2000; 870(1-2):169-177.

8. Eljaschewitsch J, Padberg J, Schurmann D, Ruf B. High performance liquid chromatographic determination of pyrimethamine, dapsone, monoacetyldapsone, sulphadoxine, and N-acetylsulfadoxine after rapid solid-phase extraction. Ther Drug Monit, 1996; 18(5):592-597.

9. Astier H, Renard C, Cheminel V, Soares S, Mounier C, Peyron F, Chaulet J F. Simultaneous determination of pyrimethamine and sulphadoxine in human plasma by high-performance liquid chromatography after automated liquid-solid extraction. J Chromatogr B Biomed Sci Appl, 1997; 698(1-2):217-223.

10. Midskov C. High-performance liquid chromatographic assay of pyrimethamine, sulfadoxine and its N4-acetyl metabolite in serum and urine after ingestion of suldox. J Chromatogr, 1984; 308:217-227.

11. Venkata Raveendra B.V., Pankaj K.S., Indrajeet S. HPLC Method Development and Validation for Simultaneous Estimation of Sulphadoxine and Pyrimethamine in Tablet Dosage Form. International Journal of Pharma Sciences. 2013; 3, (4): 295-298.

12. Sandhya S.M., Shijikumar P.S. A Simplified Liquid Chromatography-Mass Spectrometry Method for Simultaneous Determination of Pyrimethamine, Sulphadoxine and Artesunate in Human Plasma. Journal of Applied Pharmaceutical Science. 2015; 5 (06), p. 109-114.

13. Onah J O, Odeiani J E. Simultaneous spectrophotometric determination of sulphadoxine and pyrimethamine in pharmaceutical formulations. J Pharm Biomed Anal, 2002; 30(3):851-857.

14. 1S. Sharma and 2M.C. Sharma Determination of Sulfadoxine in Pharmaceutical Formulations by Dual Wavelength Spectrophotometry Using Methylene blue American-Eurasian Journal of Scientific Research , 2011; 6(4): 205-209.

15. Elbashir A.A., Elwagee A.H.E. Spectrophotometric determination of pyrimethamine (PYR) in pharmaceutical formulation using 1,2-naphthoquinone-4-sulfonate (NQS), Journal of the Association of Arab Universities for Basic and Applied Sciences. 2012; 11, 32–36.

16. Patel K.N., Patel K.J. Spectrophotometric methods for simultaneous estimation of Pyrimethamine and Sulphadoxine in bulk drug and pharmaceutical formulations (2012), Pharmatutor-art-1228.

17. Sharma S , Neog M, Prajapati V, Patel H, Dabhi D. Spectrophotometric Estimation of Sulfadoxine in Pharmaceutical Preparations. E-Journal of Chemistry, 2010; 7(4), 1246- 1253.

18. ICH G. Validation of analytical procedures: text and methodology. Q2 (R1). 2005; 1.

19. Harvey D. modern analytical chemistry, 1st ed. 2000. p. 2, 39-47, 85-95, 205-215, 369-400.